Investigating the potential of visible and near-Infrared spectroscopy (VNIR) for detecting phosphorus status of winter wheat leaves grown in long-term trial

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Abstract

The determination of plant nutrient content is crucial for evaluating crop nutrient removal, enhancing nutrient use efficiency, and optimizing yields. Nutrient conventional monitoring involves colorimetric analyses in the laboratory; however, this approach is labor-intensive, costly, and time consuming. The visible and near-infrared spectroscopy (VNIR) or hyperspectral non-imaging sensors have been an emerging technology that has been proved its potential for rapid detection of plant nutrient deficiency and nutrient status monitoring. However, most studies in this respect have focused primarily on nitrogen and few research were conducted to understand the specificity of measuring phosphorus using this technique. In this study we investigated the potential of leaf spectral reflectance in the visible and near infrared spectral region to predict phosphorus (P) status in winter wheat leaves. The research was conducted in a long-term experiment, which installed in 1896 at the Gembloux Agro-Bio Tech faculty. The trial includes various fertilization modalities ensuring phosphorus contrast and variability in data acquired. The spectra acquisition and leaves biomass sampling were done synchronously at different stages of wheat growth cycle. The reflectance measurements were done on the two youngest fully expanded leaves using the ASD FieldSpec4 spectroradiometer. The recorded spectra, between 350 nm and 2 500 nm at a 1 nm interval, were corrected for light scattering using multiple scatter correction (MSC). Results from partial *least squares regression (PLSR) with leave-one-out cross-validation (LOOCV) and 4 latent variables provided a root mean square error (RMSEcv) and a determination coefficient (R^2cv) at respectively 0.94 mg/g and 0.71. The obtained model predicted leaf phosphorus status with a ratio of standard deviation to RMSEcv (RPDcv) of 1.9. The cross-validation results showed that the developed PLS predictive model has some potential to detect P status in wheat fresh leaves by identifying 2 classes of P and that using Vis-NIR spectroscopy is a practical option to measure leaf phosphorus concentrations.*

Keywords: *Phosphorus, visible near-infrared spectroscopy, winter wheat, PLSR*

Introduction

Visible and near-infrared (VNIR) spectroscopy or hyperspectral non-imaging sensors have been an emerging technology that has been proved its potential for nutrient status detection in precision agriculture (Prananto et al., 2020). This technique relies on the change that nutrient stress can cause in the spectral reflectance characteristics of leaves and canopies indirectly by disturbing the photosynthetic pigments production such as chlorophyll and anthocyanin (Neuner and Larcher, 1990; Siedliska et al., 2021). This correlation allows a non-destructive and rapid assessment of nutrient status in the field. In contrast to stressed plants, which have a lower reflectance in the NIR, healthy crops tend to reflect a bit in the red and a lot in the NIR (Ge et al., 2019). This approach was adopted to assess nutrient crop status namely nitrogen because it is strongly correlated to the chlorophyll content that mainly affects leaf reflectance in the visible range (Wang et al., 2014). Unlike nitrogen deficiency, phosphorus starvation does not develop leaf chlorosis but it increases the number of smaller cells per unit leaf area which causes modifications in the spectral reflectance (Mahajan et al., 2014).

Several studies have been focusing on determining the appropriate wavelengths or combination of wavelengths for phosphorus (P) leaves and canopy sensing. Among several investigated vegetation indexes (VIs), Kawamura et al.,(2011) found that the NDSI, based on 523 nm and 583 nm, had the best potential to predict pasture P content (R^2=0.78). However, (Ansari et al., 2016) used the entire visible region of the spectrum to sense P in wheat during all growth stages. Similarly, (Mahajan et al., 2014) proposed a new VI that involves 1080 nm and 1460 nm wavelengths and predict P content in wheat with a significant accuracy and a correlation coefficient equal to 0.42. In the VNIR region, eight effective wavelengths were selected to predict P content in oilseed rape leaves with a high accuracy (r=0.71) (Zhang et al., 2013). Using the same region of the spectrum, (Osborne et al., 2004) found the best prediction of P in corn plants using reflectance in the blue region (440 and 445 nm) and NIR region (730 and 930 nm). The short-wave infrared domain has been also a subject of plant P prediction studies. For this purpose, Pimstein et al., (2011) suggested a vegetation index of two bands (1645 nm and 1715 nm wavelengths). The previous studies related to plant P determination used the average of different scans taken form two or three youngest leaves as a representative measurement (Ge et al., 2019). Others took measurements on the fully expanded youngest leaf (Li et al., 2006; Mahajan et al., 2014).

Despite of the several conducted studies to understand phosphorus selectivity, the obtained results were typically moderate and highly variable (Mapare et al., 2013). Therefore, further researches are needed to develop phosphorus remote sensing and to understand the specificity of measuring phosphorus content of wheat leaves using spectroscopy in order to enhance nutrients use efficiency and crop productivity. The objective of this study is to evaluate the effect of the resulting contrast of long-term fertilization modalities on wheat leaves and canopy's reflectance using the VNIR-SWIR spectroscopy for a rapid detection of P deficiency.

Material and methods

Experimental site and design

The experimental site is a long-term trial located at Gembloux Agro Bio-Tech, University of Liège, Belgium (Figure 1). This trial has been installed in 1896 with an objective to study for the long term the effect of nitrogen, phosphate, and potassium on field crop yields. The experiment was based on the law of minimum established by Liebig in 1850, which consider that plant growth and yield is limited by the element in shortest supply.

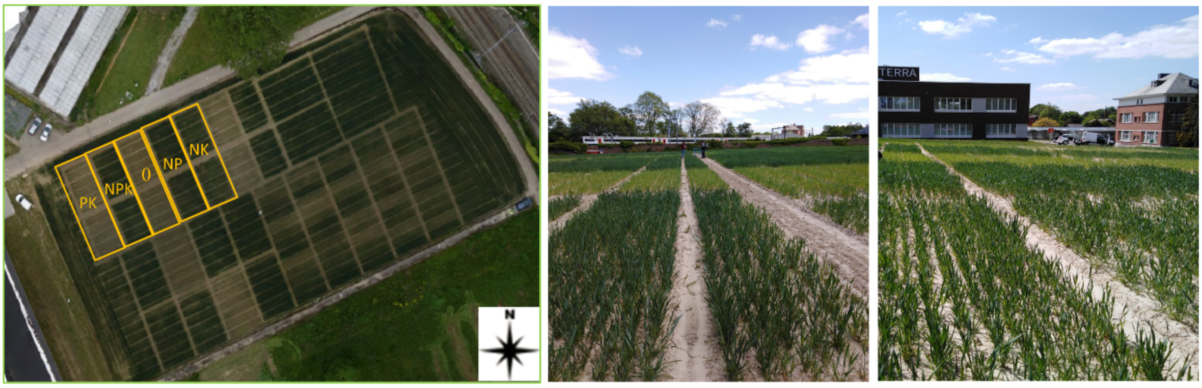

Figure 1. Photos of the region of interest with the five fertilization modalities.

The long-term trial is located in Gembloux, Belgium (50.564121, 4.698802). The trial is two sets of five plots; each plot occupies a 60m^2 area divided to 5 micro-plots with 6m length and 2m width (Figure 1). Each plot represents a different fertilization modality as it is shown in the figure. In our study, two lines of micro-plots were eliminated to avoid the border effect. Five different fertilization modalities were studied, NPK symbolizes the fertilization modality with the supply of the three 3 macronutrients (nitrogen, phosphorus, and potassium), PK is the phospho-potassium fertilization, NK is the nitrogen and potassium fertilization, NP represents the nitrogen and phosphorus fertilization, and 0 treatment is where no supply of the three macronutrients has been applied. The nutrient supplies are done at the Z21 stage according to Zadok scale for phosphorus, potassium, and the first fraction of the total amount of nitrogen, while the second and the third fractions were applied at Z30 and Z50 stages, respectively. The trial was supplied with the optimal rates of the essential nutrients K at 160 Kg/ha, P at 120 Kg/ha, and N at 150 Kg/ha using the ammonium nitrate, triple superphosphate and potassium chloride fertilizers.

Measurement of hyperspectral reflectance

The spectra acquisition was done weekly starting from flowering to maturity stages using the ASD FieldSpec4 spectroradiometer (Malvern Panalytical Ltd., Formerly Analytical Spectral Devices). The spectral range of the instrument is 350–2500 nm and the spectral sampling interval is 1 nm. Each raw spectrum therefore has 2151 data points. The acquisitions were taken between 10h00 and 15h00 at the leaf level using the contact probe of the spectroradiometer. Five plant per micro-plot were chosen randomly for the measurements and for each plant the reflectance of two fully expanded youngest leaves were recorded (the first and the second leaf from the top). For each leaf, one measurement was taken at the largest section. Acquisitions were done weekly between flowering and maturity stages, during six weeks.

Biomass sampling and chemical analysis

Biomass samples were collected using a 50 x 75 cm quadrat comprising 6 lines. The samples were taken at three development stages 69, 77, and 89 according to Zadocks scale (Zadocks et al., 1974). After the sampling work, the wheat plants were separated to leaves, stems, and ears and weighed to obtain the fresh weight of the samples. The wheat samples were then dried to achieve constant mass and weighed to record the dry weight. Nitrogen concentration, phosphorus concentration and other major elements concentration (K, Mg, Ca, Na) were determined on the same samples.

Spectra preprocessing and analysis

To remove the light scattering effect from the raw data, the MSC was applied using the "prospectr" package (**Stevens and Ramirez-Lopez, 2015)**. It consists of eliminating additive noise and multiplicative noise through the separation of physical light scattering effects and chemical light effects in spectra **(Martens and Stark, 1991).** The MSC processing technique corrects each spectrum by dividing it by its slope and subtracting its intercept; the slope and the intercept are calculated by regressing each spectrum against the average spectrum **(Geladi et al., 1985).**

Before conducting the multiple regression, the water's influences on the measured spectra were removed by eliminating the water absorption bands (Figure 2.c). In fresh plant leaves, water absorbs energy in the SWIR region particularly near 1450 and 1900 nm bands **(Peñuelas and Filella, 1998)**. Therefore, the wavelength ranges from 1350nm to 1550 nm and from 1800 nm to 2000 nm have been excluded from the spectral analysis. In addition, the wavelengths beyond the range of 400–2400 nm were also removed.

The number of samples for each value of phosphorus is presented in Figure 2.d. The leaves phosphorus content of the 60 collected samples covers all the range from 0.6 to 6.4 mg.g^{-1}. These values were used as reference measurements to establish phosphorus prediction model using partial least square regression from PLS package. Due to the moderate number of phosphorus samples, leave-one-out cross validation was adopted to train and validate our predictive model. After establishing the model, we proceeded to wavelength selection via variable importance.

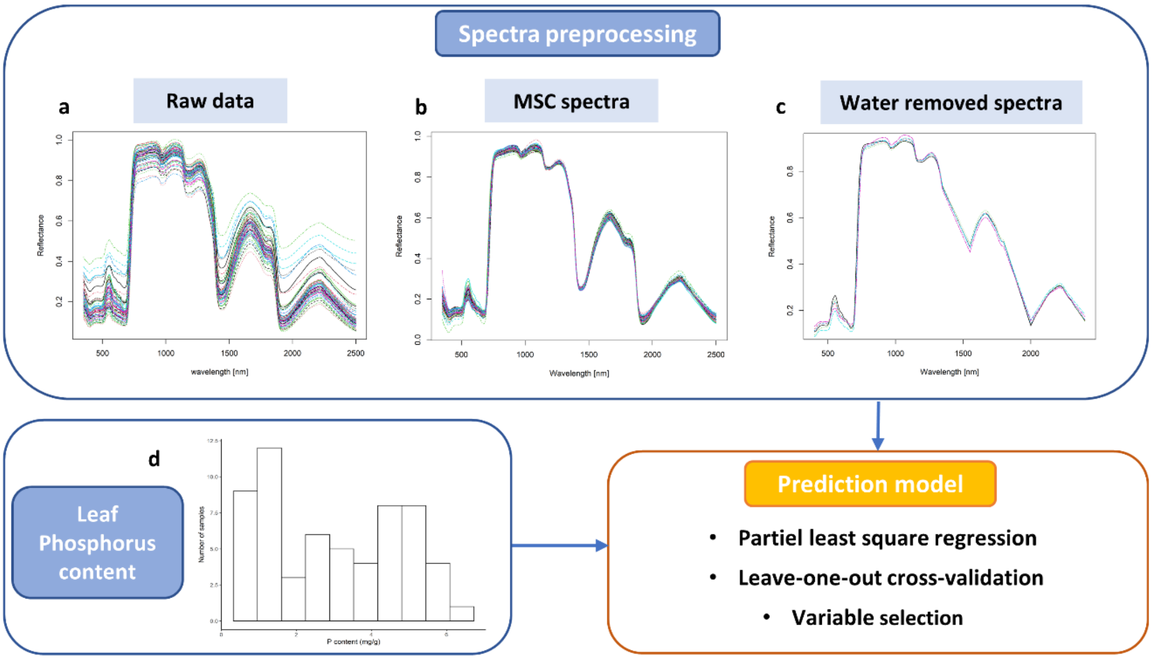

Figure 2. Raw data preprocessing workflow: raw data (a), multiple scatter corrected spectra (b), water bands retrieval (c), and reference measurement distribution (d)

Results

Averaging the spectra

Figure 3 shows the average reflectance spectra of different fertilization modalities (PK, NPK, None, NP, and NK) from the raw data and after the scatter correction (MSC) for the first leaf. Comparing the average corrected spectra of the NPK modality (presence of phosphorus) and the NK modality (absence of phosphorus) shows that decreasing phosphorus increases reflectance in the range between 800 and 1000 nm and decreases reflectance near the 1700 and 2300 nm bands (Figure 3.B).

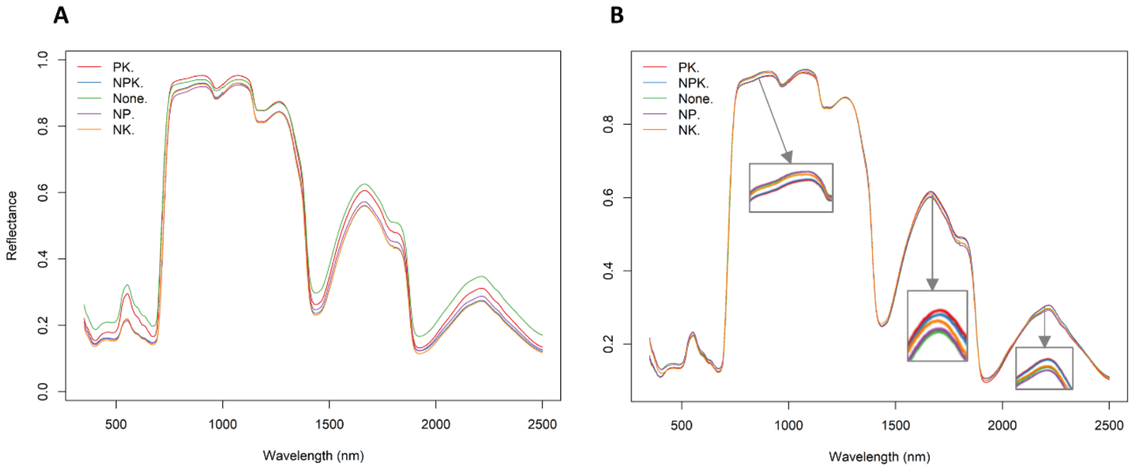

Figure 3. The average spectra of leaf samples from different fertilization modalities of raw spectra (A) and the MSC corrected spectra (B).

PLS-based prediction models

The 1603 remaining wavelengths after water bands and noise retrievals, were used to perform a partial least square regression for each leaf apart. For the two models the optimum number of latent variables was determined by minimizing the mean square error (RMSE). Using the raw data, the first variable latent explains 29.56 and 40.64% of the variation of phosphorus content in the first and second leaf, respectively. After applying the scatter correction and water band removal, this percentage increases to 61.48 for the first leaf and to 59.64% for the second leaf. For the first four variable latent, the highest percentage was recorded for the preprocessed spectra of the 2nd leaf of measurements with a percentage of 77.57%, see Table 1.

Table 1. The variance of latent variables in raw data PLS model and preprocessed spectra PLS model for each leaf.

Latent variable number	Raw data		Preprocessed spectra	
	1st leaf	2nd leaf	1st leaf	2nd leaf
1	29.56	40.64	61.48	59.64
2	45.61	65.92	63.30	69.70
3	70.42	69.87	69.27	72.81
4	74.36	75.99	74.20	77.57

Effect of leaf number on the model accuracy

For the both PLS-based prediction models, the accuracy was assessed by calculating root mean square error for cross-validation (RMSEcv), coefficient of determination (R^2), and the ratio of standard deviation to RMSEcv (RPDcv) for cross-validation (RMSE cv), The results are presented in Figure 4. This shows the observed versus predicted values of leaves phosphorus concentration (mg/g) from the final PLS leave-one-out cross-validation for the first leaf (A) and the second leaf of measurement (B). The 2nd leaf-based prediction model outperformed the first model in terms of accuracy. The coefficient of determination and RPD for the 2nd leaf-based model were around 0.71 and 1.9, respectively. Furthermore, the RMSEcv was also slightly inferior to those of the 1st leaf-based model.

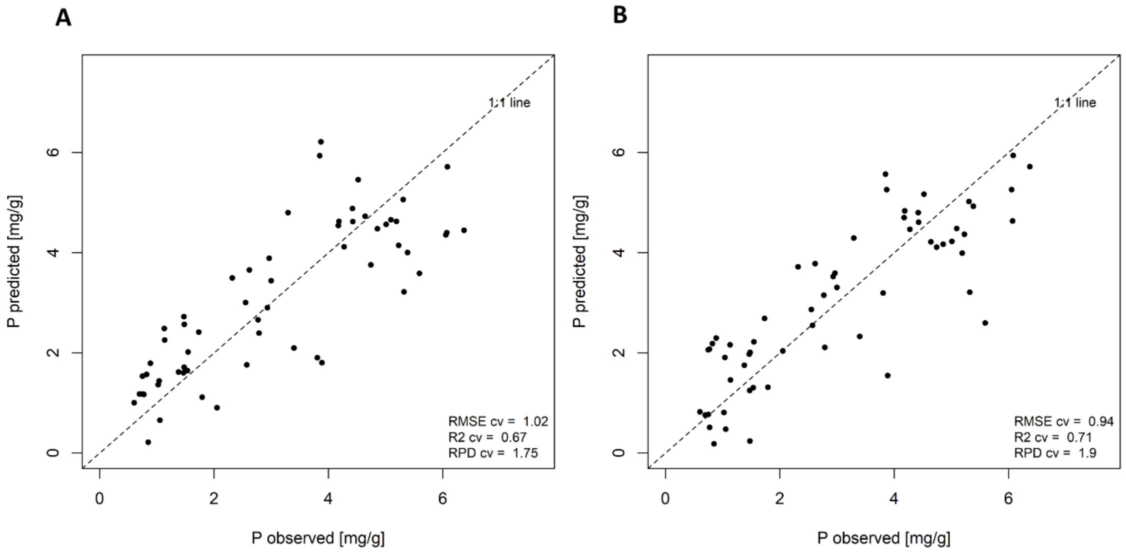

Figure 4. The observed versus predicted values from the final PLS leave-one-out (loocv) cross-validation procedure for leaves phosphorus concentration (mg/g), for the first leaf (A) and the second leaf (B) of measurement.

Variable importance

The effective wavelengths of the spectral data were selected using the variable importance in projection (VIP) scores from the PLS with the full spectrum. The VIP values for all variables and for the four latent variables are displayed in Figure 5. The four latent variables explain the same regions of the spectra, particularly in the visible region. The third and the fourth latent variables are responsible for explaining small additional variability in the region around 1500 nm. The zoom on the visible and the NIR regions of the importance plot shows two distinct peaks, the first is around 560 nm and the second at 720 nm.

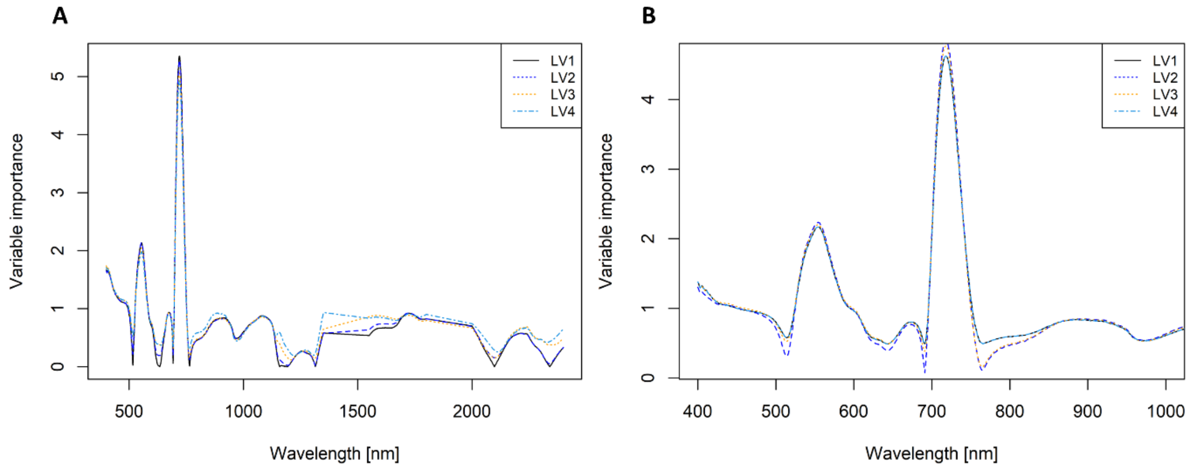

Figure 5. Variable importance plot for the first four latent variables of the second leaf PLS model over the full spectrum (A) and only the visible NIR regions (B)

Discussion

In this study, we investigated two different prediction models based on the full spectral region of two different leaves. The two PLS-based models predicted leaves phosphorus moderately and the models have some potential to be improved (R^2=0.67 and R^2=0.71 for the first and the second leaf respectively) The second PLS model had higher coefficient of determination and ratio of performance to deviation (RPD>2.0) and lower root mean square errors than the first leaf prediction model, but differences were small. (Rossel et al., 2007) used R^2 and RPD to distinguish different classes of models. R^2 between 0.65 and 0.80 and RPD between 1.8 and 2.0 indicates good models and predictions where the model can produce quantitative predictions,

The effect of the leaf number on the quality of prediction of nitrogen using reflectance was studied by (Röll et al., 2019), no significant difference was reported between the two youngest leaves while predicting N content using spectral vegetation indices. When taking spectral measurements on the youngest fully developed leaf, (Li et al., 2006) found no influence of P leaf content between control and P-deprived bailey plants. The authors recommend that spectral analysis be performed on older mature leaves since P is mobile and can be reabsorbed from older organs to young leaves. This prevents the young leaves from entering P-stressed state.

Our results showed that the VNIR spectral region was the most related to leaves phosphorus content and the most sensitive bands were around 560 nm and 720 nm, which can be related to the anthocyanin absorption bands and to the red-edge respectively. Salisbury and Ross, (1992) reported that the resulted purple coloration in phosphorus deficient leaf margins is caused by the absorption of green lights (500-600 nm) and the reflection in the red and blue regions of the spectrum. Using hyperspectral reflectance data, (Li et al., 2018) demonstrated that the red-edge bands (680–760 nm) can be utilized to accurately estimate leaf phosphorus content ($R2_{val}$ = 0.75, RPD_{val} = 2.01), which is similar to our findings. On the other hand, a low prediction accuracy for P using the full spectral range was found by (Ge et al., 2019) with R^2 < 0.5 and RPD < 1.4. Özyiğit and Bilgen, (2013) obtained a low coefficient of determination (R^2=0.43) while detecting phosphorus content using two wavelenghts of the red-edge in the equation (R675, R680). In recent studies, the Visible green region and NIR region were also found to relate to phosphorus, and the effective wavelengths for P were 416, 421, 424, 427, 458, 485, 664, 819, 828, 839, 902, and 933 nm (Peng et al., 2020). The visible spectral region is the pigments absorption region, the chlorophyll tends to absorb in the blue (400-500 nm) and red (660-680 nm) spectral regions (Meler et al., 2017). The effects of P content on maize growth and spectral reflectance were studied and the sensitive bands of P were 763 nm, 815 nm, and 900–1000 nm (Qiao et al., 2022). However, our predictive model suggests only 2 selected important wavelengths (the highest variable importance values) to predict P with high accuracy compared to newly developed spectral models.

Conclusion

The results indicates that the phosphorus leaves content impact the spectral reflectance around the wavelengths 560 nm and 720 nm. Therefore, these wavelengths could be used to detect phosphorus status in wheat leaves.

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